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Culture

Volume 22 No 1 March 2001 ISSN 0965-0989

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Chlamydia pneumoniae and atherosclerosis: association or causation

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Introduction

Atheromatous plaque formation is a pathological process involved in a number of vascular diseases including aortic aneurysm development, stroke and coronary artery disease. As such, it represents a major cause of morbidity and mortality in the industrialised world. A variety of risk factors of the development of coronary artery disease have been identified, such as smoking, hypertension, raised serum cholesterol levels and hereditary factors. Preventative regimens based on knowledge of these risk factors (eg use of serum lipid-lowering compounds) can reduce mortality associated with atherosclerotic heart disease[1]. However, known risk factors do not fully account for the incidence of atherosclerotic heart disease[2] and the mechanisms underlying formation of atheromatous plaques are not fully understood.

While the concept that infection by certain organisms may also constitute a risk factor is not new, the first evidence in support of this concept did not appear until 1978, when Fabricant *et al* [3] showed that infection of germ-free chickens with avian herpes virus led to the development of arterial lesions resembling atherosclerosis in humans. In recent years, much evidence has been presented to associate atheroma with infection. A cascade of interest in a possible link between *Chlamydia pneumoniae* infection and atheroma development was triggered by a Finnish study demonstrating that high titres of IgG and IgA antibodies to *C. pneumoniae* occurred significantly more often in males with myocardial infarction or proven coronary artery disease, than in an age-matched control population[4]. This interest was further fuelled by the subsequent visualisation of *C. pneumoniae* within atheromatous lesions by electron microscopy in 1992 by Shor *et al*[5]. Since then, while the precise figures have varied, significant serological associations between *C. pneumoniae* infection and heart disease have been demonstrated on numerous occasions in many laboratories around the world, and a variety of methods have since been used to successfully demonstrate the presence of *C. pneumoniae* within atherosclerotic lesions, including *in situ* hybridisation techniques, PCR, immunocytochemistry and immunofluorescence. Direct culture and use of reverse transcriptase PCR[7], have confirmed that organisms are not only present in atherosclerotic plaques but are also viable.

Positive findings breed related research and thus investigations have centred on *C. pneumoniae*. However, a variety of other organisms have also been associated with atheroma development, including *Helicobacter pylori*, cytomegalovirus (CMV) and herpes simplex virus type 1 (HSV-1), amongst others. Claims of an association between *Helicobacter pylori* infection and atheroma have become less credible, as the organism has not been detected directly from atheromatous lesions and meta-analysis of 18 serological studies has failed to support the association[8]. The strength of the associations between the other organisms listed and atheroma development is less clear.

Also in this issue (page 5):

Control of food-poisoning salmonellas in live poultry

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Newly hatched chick

Chlamydia pneumoniae

Characterisation on the basis of ribosomal gene sequence has led to a proposed revision of the taxonomy of the family *Chlamydiaceae*, with the species *Chlamydia pneumoniae* being reclassified within the new genus *Chlamydophila*, as *Chlamydophila pneumoniae*[6]. *C. pneumoniae* is an obligate intracellular prokaryotic pathogen. Cell culture techniques are used for isolation and propagation of *C. pneumoniae* from clinical samples. Essentially, infection of a confluent cell monolayer is achieved by centrifugation of the sample onto the monolayer and subsequent incubation at 37°C. Sensitivity of culture is increased by including an antimetabolite such as cycloheximide in the growth medium[9]. Cell types used successfully for culture include HEp-2 cells and Chang cells.

C. pneumoniae is thought to be responsible for 10% of community-acquired pneumonia[12]. The route of infection is person to person, via the respiratory tract. While infection in young children is rare, the incidence of infection increases dramatically through school years, such that by about 18 years of age, 50% of adults of both sexes have antibody to this organism. This figure continues to rise with age but with a divergence between the sexes; peaking at about 80% in men and 70% in women in old age[13] – this increased incidence in the older population, with a bias towards older males, is not dissimilar to the epidemiology of heart disease. A large body of evidence has now accumulated demonstrating a positive association between chronic infection with this organism and atheroma development, such that there can be little doubt of its veracity. However, proof that this association is causal, and that *C. pneumoniae* does not just innocently colonise human atheromatous lesions, is still lacking. Indeed, while accepting the significant association, some authors doubt that *C. pneumoniae* does have a role to play in the aetiology of atherosclerosis. For example, a recent study by Thomas *et al* [10] showed that while *C. pneumoniae* can be frequently detected within atheromatous plaques, its distribution does not correlate with either the extent or severity of lesions detected. The authors suggest that such findings are consistent with *C. pneumoniae* colonising lesions but having no role in atheroma formation and progression. However, this finding does not disprove a causal role for *C. pneumoniae* in atheroma formation and development, if chronic *C. pneumoniae* infection in fact initiates just one of a number of possible aetiological mechanisms. If the association is causal, it remains to be determined if *C. pneumoniae* infection is the trigger for atheroma development, or has a role in accelerating progression and subsequent rupture of lesions, or both.

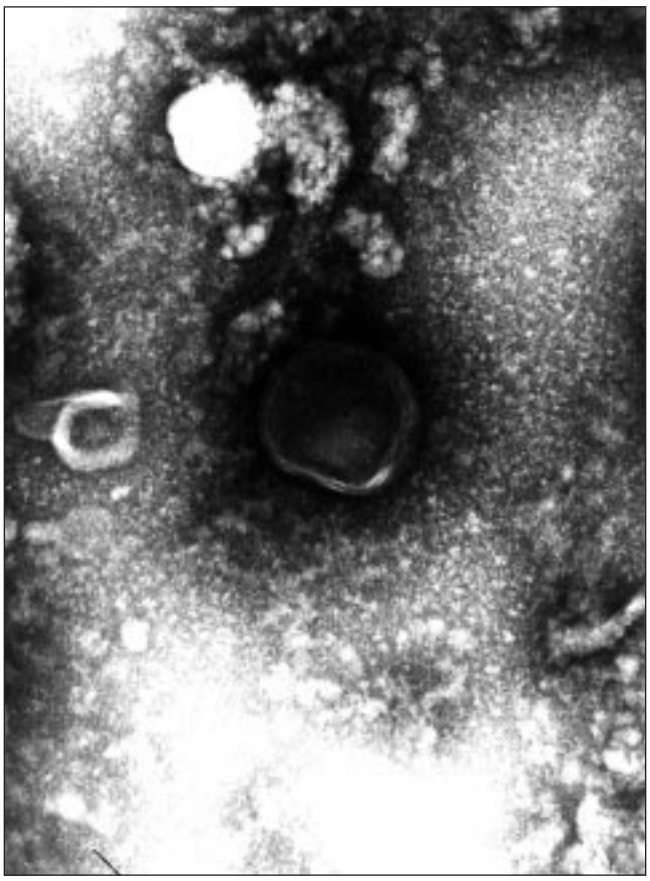

Figure 1. Electron micrograph of *Chlamydia* spp.within an atheromatous lesion. (Courtesy of Elaine Crosdale, Department of Virology, Manchester Royal Infirmary)

The question of causality

How then is the question of causality to be answered? To elucidate this issue fully, large scale antibiotic intervention trials and appropriate animal models will need to be studied. These studies must be of sufficient size to have the power to clearly demonstrate significance. Ideally, animal studies should include an uninfected, antibiotic-treated control population, to observe for any direct anti-inflammatory effects of the antibiotic used. Furthermore, the degree of benefit obtained through long term antibiotic administration in infected subjects should be correlated against the degree of antibiotic resistance determined in isolates recultured from infected lesions. Two antibiotic intervention trials that have already been undertaken reported a protective effect[14,15] while a third did not[19]. However, these trials were criticised for their small population numbers and short duration. Larger scale trials on men with proven coronary artery disease are now underway[17], the endpoints including myocardial infarction, angioplasty or hospitalisation for unstable angina. The antibiotic used is the macrolide azithromycin, chosen both for its efficacy against *C. pneumoniae* and its long half-life, permitting once weekly dosage.

These studies are of sufficient power to determine if macrolide administration can reduce the incidence of complications of vascular disease. However, such human trials will not rule out the possibility that a positive effect is due to a direct anti-inflammatory action, as opposed to an anti-infective action with indirect anti-inflammatory consequences.

One further problem with long-term antibiotic trials, is assessment of the development of macrolide resistance *in vivo*, during protracted administration. Thus, initially, animal models are better as, along with an assessment of the effect of *C. pneumoniae* infection and antibiotic administration on the cardiovascular system, they also allow for culture of *C. pneumoniae* isolates from the atheromatous

plaques of subjects, and subsequent sensitivity testing after long-term macrolide administration.

A number of animal studies of interest have already been undertaken, using both rabbit and "gene-knockout" mouse populations. "Gene-knockout" murine models have compared atheroma development in *C. pneumoniae*-infected and uninfected Apo-E deficient mouse models (a strain that spontaneously develops atherosclerosis), and demonstrated significantly larger lesions in the infected population[18]. This study suggests a role for *C. pneumoniae* infection in initiation and/or progression of atheromatous lesions. A protective role for macrolide administration has been demonstrated by a rabbit model using specific-pathogen-free New Zealand White rabbits given a small cholesterol supplement to their feed[16]. Along with a significant correlation of intranasal *C. pneumoniae* infection with extent and severity of atherosclerotic lesions, this study demonstrated that infected rabbits treated with azithromycin after inoculation showed no difference in extent or severity of atheromatous lesions to an uninfected control group, and a significant decrease in extent and severity of lesions compared with an infected, untreated population. A later study used three populations of infected rabbits given cholesterol-free feed, with one group receiving azithromycin from 5 days post-inoculation and another receiving azithromycin from 2 weeks post-inoculation; the third population remaining untreated[20]. Delayed treatment and untreated populations developed aortic atherosclerotic lesions to a similar extent and degree, while the early treatment population was significantly protected from development of atherosclerosis. This latter study suggests that early antibiotic administration is protective, and further suggests the possibility of *C. pneumoniae* infection being a "trigger" for atheroma development irrespective of any subsequent effect on lesion progression. However, both these later studies fail to negate the possibility that the effect of macrolide introduction is due to a direct anti-inflammatory effect. Thus future investigations should include a control population of uninfected rabbits receiving the same macrolide administration regimen as the infected populations. These investigations would also be of greater value if disease progression could be monitored over a greater period of time.

Another question related to the question of causality, is whether different serotypes of *C. pneumoniae* cause different patterns of disease (as is the case with other chlamydial species), with a specific strain being responsible for vascular disease. Soon after the identification of *C. pneumoniae* as a species, Campbell *et al*[11] revealed identical Southern hybridisation profiles for a collection of *C. pneumoniae* isolates, using randomly selected fragments from a *Pst* I-generated *C. pneumoniae* gene bank. Since then, only one strain of *C. pneumoniae*, the TWAR strain, has been recognised. However, recent immunoblot studies have revealed different antigenic profiles among *C. pneumoniae* isolates[21]. Also, the use of a genus-specific monoclonal antibody (Mab) directed against the Major Outer Membrane Protein (MOMP) in an immunoblot study, revealed variation in the mass of MOMPs of different *C. pneumoniae* isolates[21]. Such studies provide evidence that there are multiple serotypes of *C. pneumoniae*, and that the different antigenic determinants may lie within the MOMP.

Studies to determine if there is strain variation within the pattern of diseases caused by *C. pneumoniae* have provided varying results. Using an analysis of genomic amplified fragment length polymorphism (AFLP) fingerprints, Meijer *et al*[23] recently failed to detect any genomic differences between *C. pneumoniae* isolates of respiratory and atheromatous origin (albeit using a method designed for the genomic AFLP fingerprint analysis of *C. trachomatis* isolates). However, another study contrasting *omp*1 gene sequences (the gene coding for the MOMP) found that *C. pneumoniae* isolates of respiratory origin had identical sequences but that an isolate derived from an atheromatous lesion had sequence variations within a number of regions of *omp*1[9]. Further investigations are also required, into differences in the clinical effects of infection by atheroma-derived isolates and respiratory-derived isolates, using animal models.

Mechanism of action

If the association between *C. pneumoniae* infection and atherosclerosis is causal, by what mechanisms does *C. pneumoniae* play its aetiological role? The histological changes of atherosclerosis are thought to represent a chronic inflammatory process, possibly as a response to injury, with microbes forwarded as one possible cause of such an injury[22]. A number of sites and mechanisms of injury have been proposed. Evidence suggests that *C. pneumoniae* can infect both smooth muscle cells and invading macrophages within atherosclerotic lesions[24]. The presence of *C. pneumoniae* within smooth muscle cells is associated with cellular damage, including loss of myofilaments and accumulation of intracellular lipid. Furthermore, it has been demonstrated that incubation of macrophages in lipid leads to lipid-ingestion by macrophages, and incubation of macrophages infected with *C. pneumoniae* leads to a far greater extent of foam cell formation[25] Such changes are in accord with the histopathology of atheroma.

An intriguing mechanism of smooth muscle damage has been suggested, following the demonstration by Bachmaier *et al*[30], of cross-reaction of anti-*C. pneumoniae* outer membrane protein antibodies with cardiac-specific α-myosin heavy chain molecules sharing peptide homology, with subsequent severe heart damage in mice. Kol *et al* [26]

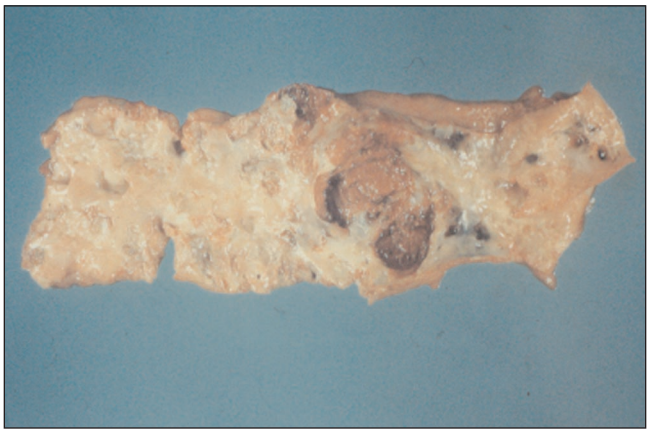

Figure 2. Atheromatous artery.

have shown that chlamydial heat shock protein 60 (HSP60) is localised in macrophages found in human atheroma and induces release of metalloproteinases and tumour necrosis factor-α; secretions relevant to the process of atherosclerosis and its complications. Other studies have demonstrated the production of chemokines and adhesion molecules in endothelial cells infected with *C. pneumoniae in vitro*[9]. These chemotactic agents would provide the stimulus for leucocyte accumulation within inflamed tissues *in vivo* – a characteristic process in atheroma development.

Conclusion

Potential aetiological mechanisms by which *C. pneumoniae* infection may lead to atherosclerosis have been investigated, and some of the studies required to determine the possibility of a causal nature to the association are underway. In advance of the results of these studies, Shor *et al*[27] have considered the likelihood of a causal association between *C. pneumoniae* infection and atheroma development, using Hill's criteria of causality[28] and their analysis is summarised here. Hill's criteria include characteristics of an association such as its strength and consistency, specificity, temporality and plausibility. The volume of significant results has suggested that the association is both strong and consistent. While organisms such as CMV are frequently found in both normal and atheromatous arteries[29], *C. pneumoniae* is rarely found in healthy vessels, fulfilling the criteria of specificity. An appropriate temporal nature to the investigation is suggested by the finding of *C. pneumoniae* in the earliest of atheromatous lesions, the fatty streak[5]. A number of factors lend weight to the plausibility of the association, such as the serological evidence and epidemiological similarities between *C. pneumoniae* infection and heart disease outlined above, and the potential mechanisms of pathogenesis that have been identified.

If the association between *C. pneumoniae* infection and atheroma development is indeed established as being causal, then the prospect is raised of using antibiotic therapy or vaccination programmes to significantly reduce morbidity and mortality associated with atheromatous vascular disease. However, in identifying an epitope of *C. pneumoniae* against which a vaccine might be developed, researchers must be mindful of the possibility of antigenic mimicry being a causative mechanism for the induction of heart disease, as described above.

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Control of food-poisoning salmonellas in live poultry

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Introduction

Human salmonellosis is a world-wide problem that has grown in recent years due to the emergence of one particular salmonella, serotype Enteritidis, as the predominant cause. Between 1979 and 1987, isolations of this organism increased in 24 of 35 countries for which information was available, and a relationship became apparent with the consumption of eggs and poultry[1].

In the live state, poultry is recognised as a major reservoir of various salmonella serotypes that cause food poisoning in man. The bacteria are found in a proportion of all healthy birds, where they colonise the alimentary tract, usually without producing any clinical sign of disease. Once acquired, the organisms are shed in the faeces and may become contaminants of products intended for human consumption. The widespread occurrence of salmonellas in poultry production has been linked to the intensive systems of management that characterise the modern industry and lead to large numbers of birds being exposed to any pathogens that happen to be present.

In relation to food safety, it is clearly important to restrict the spread of salmonellas among poultry flocks and to minimise contamination of poultry products with these organisms. Since 1990, consumption of poultry meat has steadily increased throughout the world and approximately 40 000 million chickens per year are now being produced for meat purposes[2]. The development of a global market for food and the continuing expansion of tourism highlight the growing demand for food supplies that are uniformly safe and of high quality.

This article will consider the routes by which food-poisoning salmonellas gain access to poultry, the management factors that can contribute to the problem and the different approaches now being used to reduce salmonella colonisation of the live bird.

Sources of flock colonisation

The role of feed

Traditionally, manufactured feed has been an important source of salmonellas for poultry flocks, despite measures being taken to eliminate the organisms at the mill. The problem stems from the fact that feed is invariably made from ingredients that are likely to be contaminated with salmonellas, particularly the protein component. In the UK, animal protein is required by law to be tested for salmonella and shown to be negative before it is incorporated in the feed. The legislation applies to both domestic and imported materials; however, it does not apply to the range of plant proteins and other materials now being used, such as soya bean and rapeseed meals or whole wheat, which equally may contain salmonellas. In practice, virtually every ingredient used in feed manufacture can be contaminated at one time or another, although some materials are more hazardous in this respect than others. Nowadays, feed given to poultry tends to be made only from ingredients of high microbiological quality.

Most feeds are now heat-treated to kill any salmonellas present, a process previously restricted to pelleted feed. Heat treatments vary and their efficacy depends not only on the temperature and time of heating, but also on the moisture content of the feed, which must be raised to optimise heat destruction of bacteria. An appropriate regime would be 85°C for 12 minutes in the presence of 15% moisture. Unfortunately, recontamination can sometimes occur after heat processing, especially during cooling of the feed or on subsequent handling, storage or transportation of the finished product. Air cooling, in particular, can lead to recontamination, as dust-laden air that may be carrying salmonellas is drawn over the hot feed. The problem can be minimised by using filtered air. In modern, well-managed feed mills, contamination of finished feed is normally very low. Data from the Veterinary Laboratories Agency[3] show that only 1.5% of samples from pig and poultry meals and 0.7% of those from poultry feed made by an extrusion process tested positive for salmonella in 1999. Nevertheless, even low levels of contamination are significant since newly hatched chicks can become colonised by as little as one colony-forming unit of salmonella per gram of feed. It is difficult to assess the precise role of feed in the contamination of poultry meat with salmonellas but, of the top five serotypes associated with poultry, three (Senftenberg, Mbandaka and Agona) were among the four main types isolated from feed materials[3].

Breeding flocks found to carry the invasive serotypes, Enteritidis and Typhimurium, are subject to a legal requirement for slaughter. Therefore, it is important to minimise exposure of breeder birds to salmonellas. As an additional precaution to heat processing, breeder feeds may be treated with certain chemicals, which can include formaldehyde, certain terpenes and short-chain fatty acids or their salts. Such additives serve to protect the feed from post-processing contamination, both at the mill and on the farm. The effectiveness of a product containing formic acid was demonstrated by Hinton and Linton[4]. Experience suggests that the acid is only active against salmonella when the feed has been moistened following ingestion by the bird. Consumption of acid-treated feed has no effect on an existing salmonella carrier state and the aim is simply to prevent colonisation via contaminated feed.

Transmission from breeding stock

The presence of salmonella in breeding stock can easily lead to colonisation of the progeny, a phenomenon known a vertical transmission. With those salmonella serotypes that can infect the reproductive tract of the hen, it is possible for the contents of some eggs to be contaminated directly during

the process of egg formation. Alternatively, salmonellas can penetrate the shell via faecal contamination and this route is more common. It is essential that the egg-laying environment is kept as clean as possible and eggs are collected frequently. Any visibly soiled eggs should not be incubated. As a precaution, all eggs are fumigated with formaldehyde or dipped in a chemical solution to kill surface contaminants before incubation begins.

Chicks hatch out over a three-day period and any that have acquired salmonellas from the egg can readily transmit the organisms to other chicks as they appear, especially via the fine down which is spread by circulation of air in the hatching cabinet. Salmonella colonisation in the hatchery environment can be minimised by good organisation of staff activities, management of hygiene requirements and use of sound practices for cleaning and disinfection.

The apparent affinity for the hen's reproductive tract that is shown by some strains of serotype Enteritidis has been a factor in the contamination of table eggs with this organism. Although the rate of such contamination has been low, its significance is obvious in relation to the daily consumption of some 28 million eggs in the UK. As with meat birds, statutory controls affect breeding flocks, hatcheries and feed production but the poultry industry itself has introduced a vaccination programme for layers, the relative merits of which are discussed below. The control measures have been associated with a recent decline in salmonella food poisoning in this country.

British Poultry Meat Federation

Figure..Newly-hatched chick

Salmonellas in the rearing environment

Any poultry flock may be subject to a salmonella challenge from the rearing environment, involving a variety of potential sources. These include various animal vectors: rodents, wild birds, insects and any domestic pets belonging to farm staff that are allowed to enter the rearing houses. Even farm personnel and visitors may be 'silent' carriers of salmonellas. The water supply, too, can introduce salmonellas if it is not of potable quality. There can also be a failure to clean and disinfect the house and its associated equipment properly. This will then be a source of infection for the subsequent flock. Eliminating salmonellas after the rearing of a positive flock can be difficult, since the organisms survive well outside the host, especially when present in dust particles. For those flocks that are kept in controlled environment housing, there is an opportunity to practise effective biosecurity. The concept involves control programmes for rodents and insects, exclusion of wild birds and domestic pets from the houses, and a requirement for all staff and visitors to observe good personal hygiene, eg hand washing, and to use protective clothing and disinfectant footbaths. The site itself should be clean and tidy and free from any vegetation that might harbour pests. As far as possible, vehicles should be parked well away from the poultry houses. Ideally, all flocks present on the farm at any one time should be of the same age ('all-in, all-out' stocking policy) so that the premises can be thoroughly cleaned and

disinfected when the birds have been removed for slaughter. Overall, these practices allow conditions of intensive rearing to be used to good effect in controlling pathogens like salmonella.

Use of specific intervention measures

Control of salmonellas by 'competitive exclusion'

It has long been known that commercially produced chicks are slow to develop the complex intestinal microflora of older birds because they are produced and reared initially under highly sanitised conditions, and have no contact with the mother hen. At this stage, chicks are particularly prone to salmonella colonisation due to the lack of microbial competition in the alimentary tract. Early establishment of an adult-type flora greatly increases the resistance of the chicks to a salmonella challenge[5] and is protective by a phenomenon known as 'competitive exclusion' (CE). The protection appears to be unaffected by the breed, sex or immune status of recipient birds and is active against all food-poisoning salmonellas studied so far. The flora is given orally using cultured caecal bacteria from an adult, salmonella-free donor bird. Commercial treatment products of this type are available and are tested extensively to ensure the absence of all known avian and human pathogens. Although the exact composition of these products is unknown, due to their complexity, the component organisms are merely those that would be acquired naturally by the birds in the course of time. Chicks are usually given CE treatment by means of an automatic spray-cabinet located in the chick dispatch area of the hatchery. As each tray of chicks moves through the cabinet, it receives a dose of coarse droplets that wet the upper part of the body of each bird. The chicks then preen themselves and ingest the treatment bacteria.

Protection of treated chicks is rarely complete, but the proportion of birds that subsequently become salmonella carriers is reduced, as are the levels of carriage in colonised individuals. For optimum protection, the chicks need to be salmonella-free when the CE product is administered. Salmonella colonisation is inhibited by CE treatment and the protective effect appears to involve competition between salmonellas and other organisms for receptor sites in the gut. Once established, the predominant organisms will be obligate anaerobes that produce short-chain, volatile fatty acids as metabolic end-products. Some of these acids are inhibitory to salmonellas and the effect may be enhanced by conditions of low redox potential and other factors.

CE treatment also can be used following antibiotic therapy for any disease condition or to eliminate an existing salmonella infection in older birds. Its role is to repair any damage to the gut flora that may have resulted from the medication. The combined treatment is sometimes permitted for breeding stock infected with Enteritidis or Typhimurium that would otherwise have to be slaughtered. For this purpose, the CE product is administered via the drinking water.

Vaccination against serotype Enteritidis.

In the past, vaccination has been part of the strategy for controlling infections with serotype Gallinarum, the causative agent of fowl typhoid, which has been eliminated in the more developed countries. On the other hand, the role of vaccines is less certain for many of the food-poisoning salmonellas, because little is known of the immune response elicited by strains that are non-invasive and therefore confined to the alimentary tract of carrier birds. In relation to the invasive serotype, Enteritidis, much more progress has been made and commercially prepared vaccines are available. The one licensed in the UK (Salenvac) is currently being used for more than 75% of all breeder flocks and for those laying flocks that are covered by the egg industry's Lion Code of Practice. The vaccine is an inactivated preparation for which the vaccine strain is cultured under conditions of limited iron availability that occur naturally. When grown thus, the organism expresses an iron-transfer system at the cell surface that is also antigenic and enhances the effectiveness of the vaccine. With this preparation, aluminium hydroxide gel is used as an adjuvant.

The main aim of vaccination is to prevent colonisation of the reproductive tract by Enteritidis. In doing so, direct vertical transmission is controlled and intestinal colonisation is reduced so that there is less of a hazard from the contamination of egg shells with faecal material. A further benefit is that maternal antibodies to Enteritidis appear in the egg and may help to protect the developing chick, thus limiting the spread of infection. Unfortunately, vaccination is impractical for broilers, partly because of cost and partly because each bird would require two separate injections during the rearing period. The large number of birds involved would be a problem here. For any type of bird, the treatment must be used in conjunction with other control measures, such as flock biosecurity to obtain maximum benefit. Vaccination appears to be fully compatible

Holt Studios International

Figure. White roaster chickens in large chicken house, Acampo, California, USA

with the use of CE or acid treatment of feed.

Use of an inactivated vaccine is attractive because there is no danger of any resultant infection and a good humoral immune response can be produced in the birds. In some other countries both live, attenuated and inactivated vaccine preparations are available. Possible advantages of a live vaccine are that the organism can be administered via the drinking water and pre-colonisation of chicks with one strain of salmonella tends to prevent any subsequent colonisation by another strain. Also, live vaccines may be more effective in stimulating both humoral and cellular immunity, but it is obviously important to ensure that the strains used are unlikely to revert to being virulent. Progress in the development of live, attenuated vaccines is discussed by Barrow and Wallis[6].

Conclusions

There are various sources from which poultry may acquire salmonellas during the different stages of commercial production. It is evident, therefore, that no single control measure or strategy is likely to solve the problem. Instead, appropriate controls must be implemented at all stages of production, from the egg to the final product. This article has dealt only with control strategies in the hatchery and on the farm and, since there is no means at present of achieving total elimination, control depends upon the provision of as many 'hurdles' as possible to combat survival, spread and possible multiplication of salmonellas throughout the production chain. However, differences between serotypes in colonisation potential, invasiveness and environmental persistence need to be appreciated so that controls can be targeted appropriately. Although poultry production has become increasingly intensive over the years, favouring the spread of salmonellas and other pathogens, the systems in current use do not preclude effective use of control measures, provided that there is a high level of commitment to do so on the part of management. Symptomless carriage of food-poisoning salmonellas is not a cause of economic losses within the poultry industry, but the extent to which levels of product contamination can be reduced must be dictated by cost in what is a highly competitive market. Nevertheless, public awareness of food safety issues means that there will be a continuing demand for the highest standards of salmonella control to be maintained.

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